EnzyChrom™ Maltose Assay Kit (EMLT-100)

Quantitative Colorimetric/Fluorimetric Maltose Determination

DESCRIPTION

MALTOSE ($C_{12}H_{22}O_{11}$) is a disaccharide, composed of two glucose units linked by an α bond. It is produced from the hydrolysis of glycogen or starch, serving as a source of energy for plants and animals. Maltose can be found in foods such as grains, and other processed products.

BioAssay Systems' maltose assay provides a simple, one step assay for measuring maltose. In this assay, maltose is converted to two glucoses, which are then oxidized to form a colored product. The color intensity of the product at 570 nm or fluorescence at $\lambda_{ex/em}$ = 530/585 nm is directly proportional to maltose concentration in the sample.

KEY FEATURES

Fast and sensitive. Use of 10 µL sample. Linear detection range 2 to 500 µM Maltose for colorimetric assays and 1 to 50 µM for fluorimetric assays.

Convenient. The procedure involves adding a single working reagent, and reading the absorbance after 60 minutes. Room temperature assay. No 37°C heater is needed.

High-throughput. "Add-mix-read" type assay. Can be readily automated as a high-throughput 96-well plate assay for thousands of samples per day.

APPLICATIONS

Direct Assays: Maltose in various biological samples such as serum, urine, food and beverages, etc.

KIT CONTENTS (100 TESTS IN 96-WELL PLATES)

Assay Buffer:	10 mL	**Dye Reagent:**	120 µL
Enzyme A:	120 µL	**Standard:**	1 mL 5 mM Maltose
Enzyme Mix:	120 µL		

Storage conditions. The kit is shipped on ice. Store all components at -20°C upon receiving. Shelf life: 6 months after receipt.

Precautions: Reagents are for research use only. Briefly centrifuge tubes before opening. Equilibrate all components to room temperature prior assay. Normal precautions for laboratory reagents should be exercised while using the reagents. Please refer to Material Safety Data Sheet for detailed information.

PROCEDURES

Sample Preparation: Clear and slightly colored samples can be assayed directly. It is prudent to test several dilutions to determine an optimal dilution factor *n*.

Serum can be assayed directly, after centrifuging first to remove any particulates. Appropriate dilution in distilled water may be required.

Urine samples can be assayed directly, after centrifuging first to remove any particulates, and requires the use of an internal standard.

Colorimetric Procedure

1. *Standards*. Prepare 500 µL of 500 µM Premix by mixing 50 µL of the Standard (5 mM) and 450 µL distilled water. Dilute standards in 1.5-mL centrifuge tubes as described in the Table.

No	Premix + H_2O	Maltose (µM)
1	100 µL + 0 µL	500
2	60 µL + 40 µL	300
3	30 µL + 70 µL	150
4	0 µL + 100 µL	0

   Transfer 10 µL standards into separate wells of a clear, flat-bottom 96-well plate. Transfer 10 µL of each sample into separate wells.

2. *Samples.* Add 10 µL of each sample to two separate wells in a 96 well plate (each sample requires a sample blank).

   If using an internal standard, samples will need three separate reactions: 1) sample plus standard, 2) sample alone and 3) sample blank. For the sample plus standard well, add 5 µL 500 µM maltose and 10 µL sample. For the sample and sample blank wells, add 5 µL dH_2O and 10 µL sample.

3. Prepare sufficient Working Reagent (WR) for all sample and standard wells by mixing, for each well: 95 µL Assay Buffer, 1 µL Enzyme A, 1 µL Enzyme Mix, and 1 µL Dye Reagent. For the sample blanks, prepare a Blank WR (BWR) ***without*** Enzyme A.

   Add 90 µL WR to the *four Standards* and the *Sample Wells* and 90 µL BWR to the *Sample Blank Wells*. Tap plate to mix briefly and thoroughly. Incubate 60 minutes at room temperature.

4. Read optical density at 570 nm (525-605 nm).

Fluorimetric Procedure

1. *Standards*. Dilute the standards prepared in *Colorimetric Procedure* 1:10 in dH_2O. If an internal standard is used, use 5 µL of 50 µM maltose.

2. Transfer 10 µL standards into separate wells of a black 96-well plate. Transfer 10 µL of each sample into separate wells (2 wells per sample if a standard curve is used, 3 wells per sample if an internal standard is used.

3. Add 90 µL of appropriate WR (see *Colorimetric Procedure*) to each well. Tap plate to mix briefly and thoroughly. Incubate 60 minutes at room temperature.

4. Read fluorescence at $\lambda_{ex/em}$ = 530/585 nm.

CALCULATION

Subtract the blank value (#4) from the standard values and plot the ΔOD against standard concentrations. Determine the slope and calculate the maltose concentration of the Sample,

$$[\text{Maltose}] = \frac{R_{SAMPLE} - R_{BLANK}}{\text{Slope } (\mu M^{-1})} \times n \quad (\mu M)$$

If an internal standard was used, the sample maltose concentration is computed as follows:

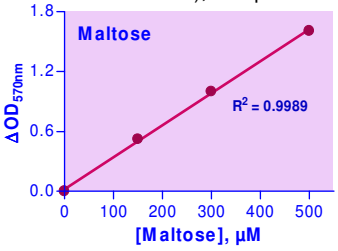
$$[\text{Maltose}] = \frac{R_{SAMPLE} - R_{BLANK}}{R_{STANDARD} - R_{SAMPLE}} \times \frac{[\text{Standard}]}{2} \times n \quad (\mu M)$$

where R_{SAMPLE}, R_{BLANK} and $R_{STANDARD}$ are OD or fluorescence readings of the Sample, Sample Blank and the Sample plus Standard respectively. *n* is the sample dilution factor. *Note: The volume of the internal standard is 2× lower than the sample volume; thus, the internal standard concentration should be divided by 2. If the calculated maltose concentration is >500 µM for the colorimetric assay, or >50 µM for the fluorimetric assay, dilute sample in dH_2O and repeat assay. Multiply result by the dilution factor n.*

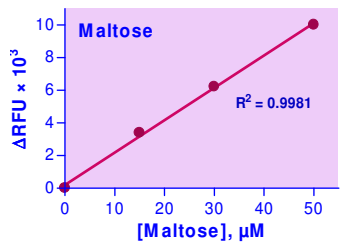
Conversions: 1 mM maltose equals 34.23 mg/dL, or 342.3 ppm.

MATERIALS REQUIRED, BUT NOT PROVIDED

Pipetting devices, centrifuge tubes, clear flat-bottom 96-well plates (e.g. VWR cat# 82050-760), and plate reader.



96-well Colorimetric Assay



96-well Fluorimetric Assay

LITERATURE

1. Young, J.M., and E. Weser (1971). The Metabolism of Circulating Maltose in Men. J Clin Invest 50: 986-991.
2. Aono, K., et al (1982). Maltose as an Energy Substrate During Surgery: Comparison of Maltose with Glucose. Can Anaesth Soc J 3: 236-42.
3. Floridi, S., et al (2001). Carbohydrate Determination in Wort and Beer by HPLC-ELSD. Monatsschrift fü Brauwissenschaft 54(9): 209-215.